the applicants' right to file one or more divisional applications directed to the deleted subject matter. In fact, the applicants firmly believe that the claims which comprise deleted claims 18 to 37 clearly relate to a single invention and may be presented in a single divisional application.

The Examiner rejected claim 13 under 35 U.S.C. 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

In this regard, the Examiner pointed out a clerical error in claim 13 with respect to the spelling of the trade mark Sephadex 6B. This clerical error now has been corrected. It is submitted that the use of trade marks in claims is permitted practice and that the claim cannot be considered to be indefinite.

It is submitted that claim 13 is no longer open to rejection under 35 U.S.C. 112, second paragraph, and, accordingly, the rejection should be withdrawn.

The Examiner rejected claims 1 to 8, 12 to 14 and 17 under 35 U.S.C. 103 as being unpatentable over Fredriksen et al in view of Jackson et al.

Claim 1 has been amended to clarify that the agglutinogen preparation which is provided by the defined process comprises fimbrial agglutinogen 2 and fimbrial agglutinogen 3 substantially free from agglutinogen 1, as specified in product claim 18. Claim 1 further has been amended to recite the manner of selective extraction of the fimbrial agglutinogens 2 and 3 from the cell paste, as previously specified in claim 6, namely by dispersing the cell paste in a buffer comprising about 1 to about 6 molar urea. In addition, the incubation step which produces a clarified supernatant containing the fimbrial agglutinogens 2 and 3 and a precipitate containing the non-fimbrial agglutinogen contaminants (agglutinogen 1) has been amended to recite the temperature range and time specified in previous claims 2 and 4.

In addition, the manner of effecting concentration of the clarified solution to produce a crude fimbrial agglutinogen

solution as specified in step (e) has been amended to incorporate the subject matter of claims 7 and 8, namely that the fimbrial agglutinogens 2 and 3 are precipitated from the clarified supernatant by the addition of polyethylene glycol, the precipitated fimbrial agglutinogens are separated from the resultant supernatant and the separated fimbrial agglutinogens 2 and 3 are then solubilized to form the concentrated solution. As a consequence, claims 2, 4, 6, 8 and 9 have been deleted and the dependency of claims 3, 5, 7 and 10 amended so as to be dependent upon claim 1.

Since claim 1 as amended incorporates the subject matter of claim 9, a claim not rejected on the basis of the recited combination of references, the rejection of claims 1 to 8, 12 to 14 and 17, insofar as they remain in the application, under 35 U.S.C. 103 as being unpatentable over Fredriksen et al in view of Jackson et al is obviated and should be withdrawn.

The Examiner had rejected claims 9 to 11 under 35 U.S.C. 103 as being unpatentable over Fredriksen et al in view of Jackson et al and further in view of Gotto. It would appear that this rejection is the one to be considered with respect to the patentability of the claims now on file with this application.

The present invention is directed to a specific process for the preparation of agglutinogen preparation which comprises fimbrial agglutinogen 2 and fimbrial agglutinogen 3 substantially free from agglutinogen 1. This process requires a combination of specific process steps as recited in claim 1. The initial step is the provision of a cell paste of the *Bordetella* strain from which the agglutinogen preparation is to be formed, such as *Bordetella pertussis* (claim 17). The fimbrial agglutinogens 2 and 3 then are selectively extracted from the cell paste by dispersing the same in a buffer comprising about 1 to about 5 molar urea which produces the first supernatant containing the fimbrial agglutinogens 2 and 3 and a first residual precipitate. The supernatant then is separated from the residual precipitate.

The supernatant then is incubated to produce a clarified supernatant containing fimbrial agglutinogens 2 and 3

and a second precipitate containing non-fimbrial agglutinogen contaminants which may have been extracted during the extraction step and comprising mainly agglutinogen 1.

The clarified supernatant then is concentrated to produce a crude fimbrial agglutinogen solution. Such concentration is effected by first precipitating the fimbrial agglutinogens 2 and 3 from a clarified supernatant by the addition of polyethylene glycol, separation of the precipitated fimbrial agglutinogens 2 and 3 from the resulting supernatant and solubilizing the separated fimbrial agglutinogens 2 and 3. This crude fimbrial agglutinogen solution then is purified, such as by column chromatography (claim 12), to produce the fimbrial agglutinogen preparation comprising fimbrial agglutinogens 2 and 3 substantially free from agglutinogen 1.

It is submitted that such a process is nowhere found in the combination of references that the Examiner has relied on.

As the Examiner correctly points out, the Fredricksen et al reference teaches purification of agglutinogen 3 from *B. pertussis*. There is no mention in this reference of the provision of a preparation containing agglutinogens 2 and 3 free from agglutinogen 1. The only disclosure in the reference is that the procedure is directed to a preparation of agglutinogen 3.

In addition, as the Examiner correctly points out, the *B. pertussis* strain is harvested. The harvested cells are resuspended in a PBS containing sodium chloride and then this suspension was added to 10 volumes of acetone to effect dehydration of the bacteria, followed by evaporation of the acetone to obtain a dry powder.

Applicants' procedures differ significantly from this prior art procedure, in that applicants effect a selective extraction of fimbrial agglutinogens 2 and 3 from a cell paste of a *Bordetella* strain by dispersing the cell paste in a buffer comprising about 1 to about 6 molar urea which then produces an aqueous supernatant which contains the agglutinogens 2 and 3 and a residual precipitate. The applicants' procedure does not

use an organic solvent, namely acetone, to effect dehydration of the bacteria as effected in the Fredriksen et al process.

According to the procedure of Fredriksen et al, the powder which results from the acetone dehydration and evaporation is resuspended in PBS and whole cells and larger fragments are removed by centrifugation. The resuspension and centrifugation are effected two more times to result in a crude extract. Applicants' procedure does not necessitate resuspension of a powdered extract nor the removal of whole cells and larger fragments from any such resuspension, but rather the initial selective extraction step by the use of a buffer comprising about 1 to about 6 molar urea successfully separates the agglutinogens 2 and 3 from the cellular fragments and hence there is no necessity for the elaborate procedure employed by Fredriksen et al to obtain his crude extract.

The Fredriksen et al procedure next heats the crude extract at 80°C for five minutes and then removes the aggregated material. Applicant effects a similar procedure by incubating the aqueous supernatant at a temperature of about 75 to about 85°C for about 10 to about 60 minutes to produce a clarified supernatant containing the fimbrial agglutinogens and a second precipitate containing non-fimbrial agglutinogen contaminants. Applicants' process involves the use of a longer period of time than Fredriksen et al and the Fredriksen et al reference does not in any way indicate that this incubation step results in the removal of the non-fimbrial agglutinogen contaminants to leave fimbrial agglutinogens 2 and 3 in a clarified supernatant.

The supernatant which results from the heat treatment step in Fredricksen et al then is subjected to an ammonium sulphate precipitation step. The precipitate is removed by centrifugation and subsequently dissolved in PBS followed by further centrifugation. In applicants' process, in contrast to this procedure, applicants precipitate fimbrial agglutinogens 2 and 3 from the clarified supernatant by the addition of a polyethylene glycol, separation of the precipitated fimbrial agglutinogens from the resulting supernatant and solubilization of the precipitated fimbrial agglutinogens to form a

concentrate.

The fraction that results from the Fredricksen et al procedure then is subjected to gel filtration chromatography for purification as in the applicants' process.

It will seem from the above analysis that applicants' process differs significantly from that described in Fredricksen et al with respect to the prior steps employed. The applicants' process is directed to the provision of and agglutinogen preparation comprising fimbrial agglutinogens 2 and 3 substantially free from agglutinogen 1. The Fredricksen et al reference is silent as to agglutinogen 2 and absence from the product of agglutinogen 1. Applicants effect extraction of fimbrial agglutinogens from a cell paste of a *Bordetella* strain by the use of a buffer containing 1 to 6 molar urea. Fredricksen et al do not effect any such process step but rather use an acetone dehydration followed by a three-fold resuspension and centrifugation.

Applicants' process involves the utilization of polyethylene glycol to effect precipitation of agglutinogens 2 and 3 from the crude preparation whereas Fredricksen et al utilize ammonium sulphate precipitation.

Applicants' process, therefore, differs significantly from the procedure which is described in Fredricksen et al both in terms of the product obtained and the procedure utilized. It is submitted that the secondary references that are relied on by the Examiner in no way remedy the basic defects of the Fredricksen et al reference.

The Examiner refers to the Jackson reference for a teaching of purification of pertactin from pertussis in which cells are extracted with urea followed by separation of the precipitate from the supernatant by centrifugation. As the Examiner correctly points out, the whole thrust of the Jackson et al reference is directed to the preparation of purified pertactin and, in one embodiment of the procedures described, pertactin is extracted from the grown cells, using urea followed by removal of cell debris by centrifugation. Ultrafiltration of the supernatant is described as being effected to remove high molecular weight proteins with

agglutinogens being indicated to be among the high molecular weight proteins which are removed by such procedure.

It is clear that the Jackson et al reference in no way suggests any further processing or procedure for separation of the agglutinogens and certainly does not disclose or suggest a procedure which is able to produce an agglutinogen preparation comprising agglutinogens 2 and 3 substantially free from agglutinogen 1. All that the Jackson et al reference is concerned with is preparing a solution from which pertactin may be separated in a pure form.

Jackson et al effect a cell extraction procedure using four molar urea and ultrafiltration to remove high molecular weight contaminants from that solution. It is submitted that such a procedure falls far short of any suggestion of a preparative procedure for obtaining agglutinogens 2 and 3 free from agglutinogen 1. At best, Jackson et al suggest that one procedure whereby pertactin may be isolated involves an initial cell extraction using aqueous urea, followed by a separation step which discards any agglutinogens and other high molecular weight proteins which may be present in the extract.

The Examiner asserts:

> "It would have been obvious to the ordinarily skilled artisan at the time the invention was made to have suspended the cell paste of Fredriksen et al. in urea in order to remove the agglutinogens from the surface of *B. pertussis* as taught by Jackson et al. instead of using the mechanical shearing process taught by Fredriksen et al. since the examiner takes Official Notice of the equivalent function of these two methods of separating cell surface proteins and the selection of any of these known equivalents would be within the level of ordinary skill in the art depending on the availability of a homogenizer or that of urea."

It is submitted that the Examiner is incorrect in this regard. The Fredricksen et al reference is concerned with the preparation of agglutinogen 3, while the Jackson et al reference is concerned with the preparation of pertactin. In fact, in the Jackson et al reference, any agglutinogens which may be present are discarded and clearly are not an intended product of the process. As already pointed out above, the

Fredricksen et al reference in fact utilizes an acetone dehydration of the cellular material followed by three successive resuspensions and centrifugations in order to remove whole cell material. Applicants' process involves extraction of cell paste using an aqueous solution of urea to selectively extract agglutinogens from the cell paste. There is no suggestion whatsoever from the combination of prior art that one could substitute urea extraction for the acetone dehydration and resuspension and centrifugation operations of Fredricksen by selective extraction of cell paste by aqueous urea in the preparation of a composition comprising agglutinogens 2 and 3 free from agglutinogen 1. The Jackson et al reference falls far short of any teaching of any such substitution, particularly since any agglutinogens which Jackson extracts in the urea extraction process are discarded and in no way form a desirable product.

The Examiner is correct that neither the Fredricksen et al nor Jackson et al reference teach the applicants' concentration step, as recited in claim 1. In fact, Jackson et al has already discarded the agglutinogens and contains no suggestion of further processing of that material nor contains any specific disclosure of any procedure for effecting any such further processing.

The Fredricksen et al reference, following the acetone extraction provision of a powder, removal of the acetone, resuspension, triple centrifugation and heat treatment then precipitates material by the addition of ammonium sulphate. Applicants' procedure does not utilize an ammonium sulphate precipitation step nor indeed the steps that Fredricksen discloses up to that point but rather applicants effect concentration of a clarified supernatant containing fimbrial agglutinogens 2 and 3 by first precipitating these fimbrial agglutinogens from the clarified supernatant by the addition of polyethylene glycol, a reagent nowhere disclosed or suggested in the Fredericksen reference, followed by separation of the fimbrial agglutinogens 2 and 3 from the resulting supernatant and then solubilizing the separated fimbrial agglutinogens 2 and 3 to form a crude fimbrial agglutinogen

solution, which then is subjected to gel filtration for further purification.

In the rejection of claims 9 to 11 under 35 U.S.C. 103, the Examiner concedes that the Fredericksen et al reference does not disclose or suggest applicants' use of polyethylene glycol in a purification step in the preparation of agglutinogens 2 and 3, separate from agglutinogen 1. As already pointed out, the Jackson et al reference is deficient in that it nowhere disclose or suggest the preparation of agglutinogen material comprising agglutinogens 2 and 3 free from agglutinogen 1, even though disclosing extraction of cellular material with urea.

The Examiner relies on the Gotto reference in an attempt to remedy the defects of the prior art. Gotto is concerned with a process for preparation of outer membrane protein of molecular weight about 69,000 daltons, namely pertactin, i.e. the same end product as the Jackson et al procedure. The Gotto reference discloses a multistep operation for producing such a product by extracting cells and utilizing a dye ligand chromatographic support for separation of the 69,000 dalton outer membrane protein. In the specific description of the procedure involved, it is indicated in column 7, lines 57 to 63 that the protein extract resulting from incubation of the cells in an extract medium is contacted with polyethylene glycol to effect precipitation of the protein extract so as to provide a precipitate fraction which is rich in the 69K protein, i.e. the product of interest. It is further indicated that other reagents commonly used to precipitate proteins, including ammonium sulphate or organic solvent, such as ethanol or acetone, may be employed. All that this passage indicates is that there are a number of reagents which have been used or suggested to be used to effect precipitation of proteins from aqueous media and polyethylene glycol happens to be the reagent of choice for the precipitation of the 69K protein from the extract material that is produced in the Gotto reference.

This disclosure in no way suggests that polyethylene glycol may be a suitable reagent for the precipitation of

agglutinogens, since the Gotto reference is entirely silent as to agglutinogens and certainly is in no way directed to a process for the preparation of agglutinogens 2 and 3 separate from agglutinogen 1 but rather, as in the case of the Jackson et al reference, is concerned with a process for producing a 69K outer membrane protein (pertactin) of *B. pertussis*. No other product from the processing of the *B. pertussis* is desired either in the Jackson et al or Gotto process. Any other material which may be present in the extract is undesirable and is removed and discarded in a procedure to obtain a purified form of the pertactin 69K protein.

In the Office Action, the Examiner states:

> "Fredriksen et al. as modified by Jackson et al. teaches ammonium sulphate precipitation rather than using polyethylene glycol. Gotto shows that PEG and ammonium sulphate are equivalent reagents for protein precipitation and may be used in protein purification schemes for *B. pertussis*."

However, as noted above, the Jackson et al reference in no way suggests any modification to the Fredricksen et al reference, since both are directed to quite different products. Similarly, while Gotto refers to polyethylene glycol and ammonium sulphate as being reagents "commonly used" to precipitate proteins, the Gotto reference is concerned solely with a procedure for obtaining pertactin (69K protein) and in no way suggests any modification of the procedure of Fredriksen et al for the preparation of a quite different protein material, namely, agglutinogen 3. It matters not that the source of the proteins is the same, namely *B. pertussis* cells, when the procedures are directed to quite different ends.

The Examiner concludes stating:

> Therefore, because these two protein precipitation reagents were art-recognized equivalents at the time the invention was made, one of ordinary skill in the art would have found it obvious to substitute PEG for ammonium sulphate."

However, whether or not this statement is correct is immaterial to consideration of the validity of the combination of the prior art, since both secondary references are directed to the

provision of a quite different material from that provided by the primary reference and, in any event, the product of the primary reference is different from the product of the applicants' process. There is no motivation provided by either the Jackson et al or Gotto references to modify the Fredricksen et al procedure for the preparation of agglutinogen 3 to provide applicants' procedure for the preparation of an agglutinogen composition comprising agglutinogens 2 and 3 free from agglutinogen 1.

Accordingly, it is submitted that claim 1 and the claims dependent thereon are patentably distinguished from the cited combination of prior art and that, accordingly, the rejections of claims 1 to 8, 12 to 14 and 17 under 35 U.S.C. 103 as being unpatentable over Fredricksen et al in view of Jackson et al and the rejection of claims 9 to 11 under 35 U.S.C. 103 as being unpatentable over Fredricksen et al in view of Jackson et al as applied to claims 1 to 8, 12 to 14 and further in view of Gotto should be withdrawn.

The Examiner had rejected claims 15 and 16 under 35 U.S.C. 103 as being unpatentable over Fredricksen et al as modified by Jackson et al as applied to claims 1 to 8, 12 to 14 and 17 and further in view of Keiff et al.

Claims 15 and 16 are dependent indirectly on claim 1 which has been modified in the above-described respects. Claim 1 incorporates the essential subject matter of claim 9, a claim which is not included in the rejection which is based upon the Fredricksen et al and Jackson et al references. Further, the Kieff et al reference in no way suggests any modification to the procedure of Fredricksen et al and Jackson et al and indeed Gotto, which would in any manner render unpatentable claim 1 as currently on file and, accordingly, the subsidiary claims 15 and 16 are also patentable over this art.

The Examiner relies on Kieff et al solely for a teaching of a vaccine composition of a protein absorbed on alum. Applicants admit that it is known in the art to absorb vaccine components on alum for the purpose of providing immunogenic compositions. Claims 15 and 16 are directed specifically to absorption of the fimbrial agglutinogen

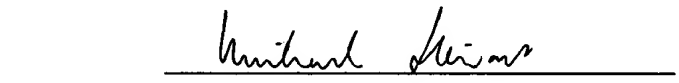
preparation comprising the agglutinogens 2 and 3 free from agglutinogen 1 on a mineral salt adjuvant, such as alum. The applicants claim no separate inventive step with respect to such recitation. However, by virtue of the dependence of these claims on claim 1, a claim which has been demonstrated to be patentable over the recited combination of prior art, it is submitted that claims 15 and 16 are not open to rejection under 35 U.S.C. 103 as being unpatentable over Fredricksen et al as modified by Jackson et al and further in view of Kieff et al and the rejection accordingly should be withdrawn.

The Examiner has cited certain additional prior art in the Office Action but do not apply such against the claims. In this regard, it is submitted that such additional prior art in no way discloses or suggests modification to the cited combinations of prior art, such as to lead to applicants' invention.

By this amendment, applicants have provided the claims in allowable form. In the event that the Examiner considers that further revisions are required to the claims in order to define the patentable subject matter thereof, the Examiner is urged to contact the applicants' representative, Mr. Michael Stewart, collect, at the number given below with a view to arriving at mutually acceptable language.

It is believed that this application now is in condition for allowance and early and favourable consideration and allowance are respectfully solicited.

Respectfully submitted,



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